HSB Project 6

Development of a Short Term Cancer Bioassay using Multiple TRP53 Haploinsufficient F1 Inbred Strains

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Background and Rationale

Genomic instability and allelic loss (loss of heterozygosity or LOH) is a hallmark of cancer and may occur early in tumorigenesis as a result of mutation and/or epigenetic alterations in response to environmental toxicant exposure. Differences between inbred strains of F1 hybrid mice for latency, tumor phenotype, and prevalence as well as nonrandom genome wide LOH were observed in preliminary studies in response to exposure to ionizing radiation (IR). Differences in DNA strand break repair (DSB) and genetic diversity in DSB repair genes have also been observed and may play a critical role in susceptibility to environmental genotoxicants and genomic instability leading to cancer.

Development of a rapid and predictive model for non-random loss of heterozygosity associated with tumor suppressor genes with sufficient genetic diversity to model the human population will enable identification of a mode of action for hazard characterization, reduce potential for false positives and false negative, and aid extrapolation of risk to human populations.

Key Issues

The B6.129-Trp53^{tm1Brd} (N12) line carries the paternal p53 null allele and the wildtype allele is of maternal origin from the strain selected for the outcross. These strains were selected based upon their genetic diversity in DNA strand break repair pathways but the DSB repair genes may not be the dominant or the only modifier of the IR induced tumor phenotype. Differences between the six different F1 progeny can be statistically analyzed for tumor latency, phenotype, and prevalence between strains for allele-allele interactions relative to the B6.129 TRP53 haploinsufficient background. F1 progeny that are statistically different from the others may be used in future studies for a F2 intercross for meiotic mapping, and those progeny carrying the Trp53 null allele phenotypes and genotyped to identify quantitative trait loci and candidate genes for further investigation.

Potential interactions in the F1 progeny between strain specific alleles, including the B6.129 Trp53 null allele, may be positive, neutral or negative and cannot be efficiently evaluated in the short-term cancer bioassay without homozygous TRP haploinsufficient controls on the same genetic background. Observed outcomes for latency, tumor phenotypes, and prevalence may thus be an average between the two strains (no epistasis) or suppressed or exacerbated by a epistatic interaction. Transcript expression analysis and allele specific loss of heterozygosity will allow investigation of allele-allele interaction for expression quantitative trait analysis (eQTL) and allele

specific loss of heterozygosity associated with significant differences in latency, tumor pathology, and prevalence. Epistatic interactions of this nature may be one reason that genome wide association studies have not been reproducible

Hypotheses

(1) Tumor spectrum, prevalence, and latency, (2) transcript or metabolomic expression profiles, or (3) expression profiles (corroborated by copy number variation) will segregate according to the haplotype of p53 haploinsufficient F1 hybrid isogenic lines selected on the basis of genetic variation in DSB repair genes.

Approach and Specific Aims

Outcross between female (A/J, BALB/C, BTBR.T/J, C3H/HeJ, DBA2/J, or 129S1.SvlmJ) and male B6.129-*Trp53*tm1Brd N13 homozygotes was used to produce F1 heterozygous progeny (AB6F1-*Trp53*^{tm1Brd}, TB6F1-*Trp53*^{tm1Brd}, BB6F1-*Trp53*^{tm1Brd}, C3B6F1-*Trp53*^{tm1Brd}, D2B6F1-*Trp53*^{tm1Brd}, 129B6F1- *Trp53*^{tm1Brd} male and female *Trp53* heterozygotes). The breeding strategy is designed to produce the desired F1 hybrid for each strain to introduce allele specific genetic variation while holding the B6 strain with the paternal null allele in common to all outcross progeny. Thus, the Trp53 wildtype allele is introduced on the maternal chromosome 11 of the outcross. Each isogenic strain was selected based upon the genetic diversity in DNA strand break repair pathway estimated by similarity matrices of SNP variants observed in the major components.

The following aims are being carried out to test these hypotheses. Six different isogenic lines (A/J, BALB/C, BTBR.T/J, C3H/HeJ, DBA2/J, or 129S1.SvlmJ) that harbor significant genetic variation in DSB repair pathway genes were outcrossed with B6.129-*Trp53*^{tm1Brd} N12 heterozygous inbred mice and their F1 progeny exposed to 0, 3, or 6 Gy ionizing radiation. The strains were selected by comparing all known DSB repair gene sequences in the 15 NIEHS/NTP-Perlegen re-sequenced strains for comparison to the C57BL/6J reference sequence and the haplotype diversity in DSB repair genes determined. Survival, tumor phenotypes, tumor and normal tissue transcript, metabolite expression profiles, and allele specific loss (loss of SNP or CNV genotypes) will be used to identify quantitative trait loci (QTL) and causally related genes and a comparative genetic analysis used to identify orthologous human genes that may predict presumed human risk to IR induced cancer.

The following data will be collected following exposure to 0, 3, or 6 Gy of ionizing radiation.

- Tumor spectrum (histopathology) and prevalence across F1 hybrid strains and haplotype.
- DNA isolated/evaluated from normal and tumor tissue or tumor allele specific copy number variation (CNV, gene deletions, and segmental duplications) and confirmation of heterozygous genotype at tumor presentation (moribund) or target tissue at termination (39 weeks post-exposure).

- RNA/isolated/evaluated from normal and tumor tissue specific transcript abundance across F1 hybrid strains and genotype at tumor presentation (moribund) or target tissue at termination (39 weeks post-exposure).
- Tissue specific protein expression across F1 hybrid strains and genotype at tumor presentation (moribund) or target tissue at termination (39 weeks post-exposure.
- Blood metabolomics profiles across F1 hybrid strains and genotype at tumor presentation (moribund) or target tissue at termination (39 weeks post-exposure).

Specific Aims

To test these hypotheses, the following aims will be carried out to produce the necessary F1 progeny and to test the null hypothesis outlined.

Aim 1. Female A/J, BALB/C, BTBR.T/J, C3H/HeJ, DBA2/J, or 129S1.SvlmJ isogenic mice will be out-crossed to heterozygous null B6.129-*Trp53*^{tm1Brd} $\geq$ N12 inbred male mice to produce the desired F1 hybrid for each strain in order to introduce allele specific genetic variation at each genetic locus.

Aim 2. Each of the F1 hybrid p53 haploinsufficient strain progeny will be exposed to a single dose of 6 Gy ($\pm$10% total dose) ionizing radiation by NIEHS investigators to induce DNA strand breaks and DNA damage and tumorigenic response in the hematopoietic target tissues.

Aim 3. Perform gross necropsy on all F1 hybrid mice observed moribund, tumor bearing, or at termination of the study and collect blood (at exsanguination) and samples from target tissues and gross malignant tissue for histopathology, DNA and RNA analysis, and metabolomics.

Aim 4. Determine the prevalence of tumor phenotypes, tumor latency, genome wide allele loss, including the *Trp53* wildtype allele, genome-wide CNV, mRNA transcript abundance, and serum metabolomic differences between tumor and non-tumor bearing mice following radiation exposure will be determined in the target tissue from mice found moribund and at 39 weeks post-exposure (termination of the study).

Aim 5. Identify an independent set of quantitative trait mouse genes determined by statistical association between biomarkers of effect (tumor prevalence, latency, transcript abundance, protein, and metabolites) induced by ionizing radiation for prediction of human orthologous gene haplotype dependent susceptibility.

Aim 6. Perform bioinformatic and comparative genomic analysis of mouse and orthologous human genes to determine if similar structural and allelic variants (SNP and/or CNV) exist and predict human risk for susceptibility to ionizing radiation-induced tumors.

Aims 1-4 are to be carried out under the NIEHS Molecular Oncology and Toxicology contract to obtain the raw data for gross pathology, histopathology, transcript and gene

copy number expression profiles by microarray and array comparative genomic hybridization, and serum metabolomics. Aims 5 and 6 will be undertaken by HSB scientists upon receipt of the raw data from ILS to NIEHS.

Significance and Expected Outcomes

Preliminary data in C3B6F1 p53 haploinsufficient mice have shown a non-random allele specific loss associated with *Melm3*, *Trp53*, and *Rad51c* genes. The males and female F1 progeny of six F1 strains have been produced and irradiated or sham irradiated. Four of the six strains have been completed and the remaining two will be completed by December 2009. To date, survival and tumor phenotypes observed by histopathology are significantly different between the four completed F1 hybrid mice studies.

Current Activities and Future Plans

These data will be analyzed to determine the value of short-term cancer bioassays to test hypotheses based outcomes with NTP-nominated chemicals. Ionizing radiation was used as an environmental exposure to test the hypothesis outlined. If successful, the results may indicate that F1 p53 or other pathway deficient strains can be selected for short-term cancer bioassays based upon genetic diversity and the predicted outcome for specific toxicity pathways. Future plans may include testing the refined model using F1 hybrids with a short-term cancer bioassay with model genotoxic and non-genotoxic NTP chemicals, if the hypotheses described here are proven. Also, strains that show significant differences can be used for F1 outcross and F2 intercross for meiotic mapping approach to produce progeny for phenotyping and genotyping for association studies to identify QTLs and candidate genes. Furthermore, both wildtype and F1 wildtype strains for short-term (28 or 90 day) exposures to identify intermediate phenotypes for predicting carcinogenic potential.

References

Pritchard, J. B., French, J. E., Davis, B. J. & Haseman, J. K. The role of transgenic mouse models in carcinogen identification. Environ Health Perspect 111, 444-54 (2003).

Boley, S. E. et al. Loss of p53 in benzene-induced thymic lymphomas in p53+/- mice: evidence of chromosomal recombination. Cancer Res 60, 2831-5 (2000).

French, J., Storer, R. D. & Donehower, L. A. The nature of the heterozygous Trp53 knockout model for identification of mutagenic carcinogens. Toxicol Pathol 29 Suppl, 24-9 (2001).

French, J. E. Identification and characterization of potential human carcinogens using B6.129tm1Trp53 heterozygous null mice and loss of heterozygosity at the Trp53 locus. IARC Sci Publ, 271-87 (2004).

Gatz, S. A. & Wiesmuller, L. p53 in recombination and repair. Cell Death Differ 13, 1003-16 (2006).

French, J. E. et al. Loss of heterozygosity frequency at the Trp53 locus in p53-deficient (+/-) mouse tumors is carcinogen-and tissue-dependent. Carcinogenesis 22, 99-106 (2001).

Schadt, E. E. et al. Genetics of gene expression surveyed in maize, mouse and man. Nature 422, 297-302 (2003).

Estivill, X. & Armengol, L. Copy number variants and common disorders: filling the gaps and exploring complexity in genome-wide association studies. PLoS Genet 3, 1787-99 (2007).

Graubert, T. A. et al. A high-resolution map of segmental DNA copy number variation in the mouse genome. PLoS Genet 3, e3 (2007).